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NUTRITION AND HEMATOPOIESIS

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Although remarkable progress has been made during recent years in the study of compounds with hematopoietic activity, the precise mechanism of action of these substances has not been explained. Nevertheless, our knowledge of the biologic properties of such substances as vitamin B_{12} (the cobalamins) and derivatives of pteroylglutamic (folic) acid, particularly the citrovorum factor, is increasing so rapidly that clearer understanding of their independent but inter-related roles is certain to be forthcoming.

It may be pertinent to emphasize at once that both vitamin B_{12} and a substance derived from folic acid are involved critically in the metabolic functions of a wide variety of cells. That these substances are often referred to as hematopoietic agents presumably is a reflection of the very great regenerative activity of tissues concerned with the formation of blood, and, accordingly, of the particular dependence of hematopoietic tissues upon these essential factors. Thus, in the average human being about 300,000,000,000 red blood cells must be manufactured daily to replace those which disappear (the average life of the erythrocyte in man is only about 120 days) (1).

Theories Concerning the Effectiveness in Pernicious Anemia of Either Pteroylglutamic Acid or Vitamin B_{12}

The widespread needs for derivatives of folic acid or vitamin B_{12} of human tissues other than those concerned with hematopoiesis, is clearly demonstrated in patients with pernicious anemia in relapse given either of these compounds. Whereas at least a week is required before any significant changes in the number of circulating red blood cells occur, the improvement in the well-being, strength and appetite of such patients, which can be observed within a day or two, is a most impressive indicator of the extra-hematopoietic functions of these factors.

An unresolved mystery is found in the fact that these several effects, so commonly seen when pernicious anemia is treated either with pteroylglutamic

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*See Page No. 16.

In this article the term *folic acid* is applied generically to those compounds related to pteroylglutamic acid which are able to promote the growth of *Lactobacillus casei* (ATCC 7469) and *Streptococcus faecalis* R (ATCC 8043); and also to support the growth of animal species *(e.g.,* the rat). Accordingly, this class of compounds includes the citrovorum factor (CF; folinic acid), which supports the growth of *Leuconostoc citrovorum* (ATCC 8081); the growth of this latter organism (under assay conditions) is not stimulated by pteroylglutamic acid (PGA), a term restricted to a specific chemical entity.

For additional references the reader is referred to recent reviews (1).

acid (PGA) or vitamin B_{12}, can be induced qualitatively as effectively with the one compound as with the other. It is true that milligrams of PGA are required, where only micrograms of injected vitamin B_{12} are necessary, but the type of clinical and hematopoietic response induced is the same with either substance. Only in one respect is there known to be an important difference: PGA, unlike vitamin B_{12}, fails to arrest certain degenerative changes in the nervous system which may be seen in pernicious anemia.

In general, there are two schools of thought concerning the functional relationship between B_{12} and a derivative of folic acid. One theory suggests that each is involved quite independently, although perhaps concerned with the catalysis of sequential reactions in certain biochemical transformations (thus, both vitamins appear to be concerned with the conversion of glycine to serine). The other concept may be regarded as permitting the inclusion of this view, but has the additional feature of posulating that, in man at least, there is also an involvement of vitamin B_{12}, directly or indirectly, in the formation (or prevention of inactivation) of a catalytically active derivative of folic acid. This second concept is proposed, of course, in order to account for the fact that in pernicious anemia there must be a deficiency of functional compounds derived from folic acid as well as of vitamin B_{12}. The disease itself is regarded as the result of a gastric defect with a consequent diminution in the secretion of various substances normally formed by the gastric mucosa, particularly intrinsic factor. As a result of the inadequacy in the amount of the latter, a failure to absorb even minute amounts of vitamin B_{12} results, as has been shown by studies with the radioactive vitamin (2). Since there is no evidence that either the amount ingested or the absorption of folic acid is significantly altered in pernicious anemia, it is concluded that the diminution in the supply of functional compounds which must be derived from folic acid is secondary to the known deficiency of vitamin B_{12}. Further, the restoration of normal function by the daily parenteral administration of only a microgram or two of vitamin B_{12}, without specific administration of PGA, also indicates that there is a resultant restoration in the efficiency of the metabolic handling of the small amounts of folic acid derived from the food and perhaps also from bacterial synthesis in the intestinal tract.

The fact that PGA in doses of several milligrams daily (or several times that apparently ingested normally) is efficacious in pernicious anemia, in spite of the postulated defect in its conversion to functional compounds, may mean that these relatively large amounts, by saturating a defective system, enable it to form adequate amounts of a functionally utilizable derivative of folic acid. According to this view, a sufficiently severe deficiency of vitamin B_{12} might lead to a failure of folic acid to induce hematopoietic remissions, and, in fact, some evidence that this is the case has been presented (3).

On the other hand, the role of vitamin B_{12} in the metabolism of folic acid in man may be to remove the effect of a substance which in some way inhibits the action of a derivative of folic acid, as has been postulated by Callender and Lajtha (4). The important studies of these workers will be given attention in a later section of this article.

Another indication that a folic acid-derived compound is the substance, rather than vitamin B_{12}, which is primarily concerned with the normal hematopoietic function of the bone marrow, is the occurrence of certain megaloblastic anemias (e.g. in pregnancy, in sprue, and in certain diseases of infants) which have been shown consistently to respond to PGA, but which are but rarely affected by vitamin B_{12}. Also, in swine, megaloblastic anemia was produced by a deficiency of folic acid and cured by the administration of PGA (5, 6), while in a deficiency of vitamin B_{12} in this species a normocytic rather than a megaloblastic type of marrow developed (6).

Conversion of Pteroylglutamic Acid to Citrovorum Factor

In view of the now convincing evidence that pteroylglutamic acid, prior to acquiring biologic activity, must be converted enzymatically to citrovorum factor (CF; folinic acid), i.e., a substance with activity for the micro-organism *Leuconostoc citrovorum* (7), it was at first thought possible that the formation of this factor might be defective in pernicious anemia. However, several workers found no serious aberration in the formation of CF from orally administered PGA, as indicated by the comparative urinary excretion of CF by normal subjects and by patients with pernicious anemia. Most recently, Spray and Witts (8) reported that the urinary output of CF, following test doses of 5 mg. of PGA, was very slightly reduced in untreated pernicious anemia (less than 2 μg. as compared to 6.9 μg. in non-anemic subjects), as also was the urinary elimination of folic acid (0.72 mg., as compared to 1.18 mg. in non-anemic subjects). However, as these workers suggested, these small changes very likely reflect the greater retention of derivatives of folic acid by the tissues of patients with pernicious anemia, rather than a true deficiency in the formation of CF. Further evidence that the formation of CF from its precursors is not significantly disturbed in pernicious anemia is afforded by the fact that the activity of parenterally administered synthetic CF in pernicious anemia is not greatly different from that of PGA. Evidence that this is the case has been discussed elsewhere (1).

In a previous issue of this review (9), published in September 1951, the status of our knowledge of the citrovorum factor at that time was presented. In addition to what has been said already in this article, or in the earlier review (9), attention may be directed to the increasing evidence for the par-

ticipation of ascorbic acid in the formation (and stabilization) of CF. The possibility of a physiologic interrelation between folic acid and vitamin C was first suggested by the observation that coadministration of the two substances either to rats or to man gave a consistent (about three-fold) increase in the urinary output of CF, as compared to the yield obtained when PGA alone was given (10). Study of this phenomenon was facilitated greatly by the discovery that PGA can be converted enzymatically to CF by liver slices *in vitro,* and that in this system the yield of CF can be augmented markedly by the addition of sodium ascorbate (7). More recent studies (11) have demonstrated that the enzyme system functions efficiently under anaerobic conditions in a cell-free extract of liver, although even under these conditions ascorbic acid augments the amount of CF formed from PGA. Since CF formed anaerobically is rapidly inactivated by this liver system under aerobic conditions, in the absence of added ascorbate, it appears that vitamin C may be involved importantly in stabilizing CF. Conceivably, ascorbic acid influences an equilibrium between CF and a related compound.

Clinical Significance of the Participation of Vitamin C in the "Activation" of Folic Acid

The importance of the role of ascorbic acid in the metabolism of CF also has been shown convincingly by the studies of May and his collaborators (12, 13, 14) in monkeys. In this species, which like man does not have the ability to synthesize vitamin C, a megaloblastic anemia gradually developed when the animals were allowed to develop scurvy. Without otherwise affecting the scorbutic state, PGA in doses approaching one milligram, restored the capacity to manufacture red blood cells; however, CF exerted the same effect in doses of less than 0.01 milligram. On the other hand, if vitamin C were given, thereby correcting all manifestations of scurvy, the anemia also was corrected promptly. It is difficult to explain these observations in any way except to suggest that with minimal intake of its precursors, CF cannot be formed in adequate amounts, or protected from inactivation, when the concentration of ascorbic acid in the tissues falls below a critical level.

In the scurvy of human adults two studies (10, 15) have shown that test doses of PGA failed to increase the urinary excretion of CF in the manner observed in normal individuals. In fact, even after the administration of large doses of vitamin C the excretion of CF in the urine rose but little (with the same test doses of PGA), and only very gradually improved with the continued administration of vitamin C. Although it is possible that a prolonged deficiency of vitamin C may lead to a change in the system concerned with the formation of CF that only gradually can be restored to normal, it is also

possible that time is required to saturate the depleted tissues with CF and its derivatives.

As pointed out by May and his associates (13), a large percentage of the reported cases of megaloblastic anemia of infancy have been seen in children in which the intake of ascorbic acid was suboptimal. Although of circumstantial significance only, it is of much interest that this condition in infants apparently has almost entirely disappeared since proprietary substitutes for human milk have been supplemented with vitamin C.

Effect of CF on Nomenclature; Composition of Tissue Forms of "Folic Acid"

An interesting by-product of our advancing knowledge of the significance of citrovorum factor is the difficulty in continuing the present synonymous use of the terms "folic acid" and pteroylglutamic acid. Whether the latter compound, which was isolated from liver (16), exists, as such, in natural materials, is not clear. There seems to be little doubt that of the substances of the folic-acid-type (see footnote at beginning of article) which are present in tissues, a very large part is CF. In turn, tissue-CF probably exists primarily as a complex in which the molecule may be associated with six γ-linked glutamic acid moieties. Whether PGA, and its conjugated form (pteroylhexa-γ-glutamylglutamic acid), each of which was obtained from natural sources, were formed entirely from CF and related compounds during the processes of isolation, remains to be determined. However, compounds with activity for *Streptococcus faecalis* and *Lactobacillus casei* (organisms used for the determination of PGA), but with little or no activity for *Leuconostoc citrovorum,* can be demonstrated in natural materials. Of interest are such substances as isoleucovorin chloride and anhydroleucovorin A, formed from synthetic CF (leucovorin) by treatment with HC1 at pH 1.3 and pH 2, respectively (17). These compounds are tetrahydro-pteroylglutamic acid derivatives in which a new ring has been formed involving the N-formyl group which characterizes CF. Although these substances behave like PGA and not like CF in microbial assay systems, they may be more readily converted to CF than is PGA, since CF treated at pH 2 for 24 hours was about one-tenth as active as synthetic CF in protecting mice against the lethal action of the PGA-analogue, aminopterin (18).

The instability of CF in the presence of acid suggests that under normal conditions of gastric acidity considerable loss of activity may occur. Attention also should be drawn to the finding that the synthetic form of CF (leucovorin; folinic acid-SF), with which much work has been done, is only one-half as active as the naturally occurring compound. By many this is thought

to be attributable to the presence in the synthetic material of two isomers, one of which has little or no activity (for discussion, see (1)). The practical significance of this finding relates to the fact that a correction factor will have to be applied to most of the studies in the literature in which the reference standard was synthetic CF.

Mechanism of Action of Aminopterin and Other 4-Amino Analogues of PGA

There has been much progress in our understanding of the rather remarkable action of the 4-amino analogues of PGA (Aminopterin, A-methopterin, etc.). That study of the mechanism of action of drugs may offer practical as well as philosophical rewards is well-exemplified by these investigations. At first the violently toxic action of these substances offered something of an enigma, since unlike ordinary competitive antagonists, their activity was but little affected by PGA, the compound which they resemble so closely in structure. It is now clear that this relative inactivity of PGA is accounted for by the fact that these analogues profoundly block the enzymatic conversion of PGA to CF (19). Apparently, the affinity of such compounds for a component of the enzyme system is such that, under physiologic conditions in animal species, sufficiently high concentrations of PGA significantly to displace the analogue cannot be obtained (except at barely effective levels of analogue-activity). However, *prior* treatment with PGA, an hour or more before administration of the analogue, is rather markedly protective. This protection can be attributed to the fact that within an hour following the administration of PGA the concentration of CF in the tissues is increased markedly (11). Against CF, in contrast to PGA, the 4-amino analogues of PGA function essentially as competitive antagonists. Consequently, protection against the toxic effects of these agents can be obtained either by prior treatment with PGA, or by the coadministration of CF, or even, under certain conditions, by subsequent treatment with CF after toxic effects have developed. Practical use of this ability of CF not only to give protection against, but also to reverse, the toxic actions of Aminopterin and its derivatives has been made in human subjects (20, 21). When, however, relatively large (lethal) doses of the analogues are given to animals, and the use of CF is too long delayed, changes occur which apparently cannot be reversed by CF (22, 23). It has been suggested that under these conditions an enzyme concerned with the conversion of CF to another substance on the pathway to the formation of a functional compound is inactivated (22).

Since the use of Aminopterin and its derivatives in the treatment of certain acute leukemias of childhood has been of considerable value in delaying the progress of the disease, although never curative, and because undesirable

side-effects are not infrequently produced, it is important to have available a compound (CF) of potential value in relieving undesirable manifestations of the powerful action of these drugs. It should be emphasized at this point that there is no evidence that CF is of any value whatsoever in the treatment of leukemia, as has sometimes been implied. Mention may be made also of a new application of these analogues: the introduction of therapeutic abortions at an early stage of pregnancy (24).

All the effects of the analogues on tissues, normal, neoplastic or embryonic, can be attributed to a profound interference with chemical reactions (in which a derivative of CF must play a catalytic role), concerned with the function and particularly the reproduction of cells. Thus, these substances can produce not only a profound interference with the formation of leucocytes, normal or abnormal in origin, and with the rapidly-growing tissues of the embryo, but also with the mucosal tissues lining the alimentary tract and with the hair-forming cells, in each of which the formation of new cells is carried on continuously at a high level. It is presumably for this reason that the analogues may produce loss of hair, as well as ulceration of the oral and intestinal tissues, and hematopoietic arrest. That anemia is not seen in man when these analogues are administered is a reflection of the fact that less than one per cent of the circulating red blood cells are lost daily and opportunity usually is not afforded for a significant degree of anemia to develop.

That megaloblastic anemia does, indeed, develop when there is a chronic rather than an acute deficiency of functional derivatives of folic acid perhaps may be attributed to the greater sensitivity of the erythrocyte-forming cells than of other tissues to a suboptimal concentration of derivatives of the vitamin. That other tissues may suffer as well, however, is seen in the profoundly disturbed intestinal function, which, in addition to megaloblastic anemia, is characteristic of sprue.

Mechanism of Action of "Folic Acid" in Catalyzing Biologic Reactions

The biochemical reactions with which a derivative of folic acid appears to be concerned are many. In essence, however, the transfer of a one-carbon intermediate (or intermediates) seems to be the common feature of these reactions. Through the use of isotopically labeled compounds a participation of folic acid has been shown in the formation of serine (3 carbon atoms) from glycine (2 carbon atoms) (here the one-carbon donor may be formic acid or another molecule of glycine) (25, 26, 27); of methionine-methyl (and thus of choline-methyls) from homocysteine and formate or methanol

(28, 29); of the methyl group of thymine from formate, glycine or the β-carbon of serine (30, 31); and of the 2-and 8 carbons of the purine ring from formate (32, 37). Buchanan (36) has described a system (in pigeon liver) in which the 2-carbon of the purine derivative inosinic acid (a precursor of nucleic acids and of uric acid), exchanges rapidly with C^{14}-formate *in vitro*. Since a participation of CF in this "inosinic transformylase" system could be shown, Buchanan has referred to the cofactor derived from folic acid as "coenzyme F." In addition to formate and glycine (the α-carbon), all the compounds listed above which are the products of the donation of one-carbon moieties, in turn may serve as donors of this group; and other compounds, such as threonine, histidine, tryptophan, choline and betaine, also can donate a carbon for these interconversions.

The precise mechanism of the participation of a CF-derived compound in these transformations has not been explained. An attractive theory suggested that the formyl group of CF (5-*formyl*-5, 6, 7, 8-tetrahydro-pteroylglutamic acid) might be shuttled back and forth by an enzyme in which the prosthetic group could be regarded as containing alternatively CF and the unformylated compound (5, 6, 7, 8-tetrahydropteroylglutamic acid). Against this view, however, Elwyn *et al* (38) have arrayed an impressive body of evidence. Their work indicates that in the synthesis of the labile methyl group (as found in choline) from the β-carbon of serine, the intermediates concerned do not involve a compound at the oxidation level of formic acid. Since folic acid *is* involved in this reaction, and because the formyl group of CF *is* at such a stage of oxidation, CF, as we now know it, appears not to function as a carrier of the "formyl" group. The solution to this new problem has not yet been found. Possibly there is another form of CF, in which a labile hydroxy-methyl group (R-CH_2OH) occurs, or possibly CF functions in an entirely different manner (e.g., as a hydrogen carrier) in catalyzing the transfer of one-carbon moieties.

Role of Vitamin B_{12} in Biochemical Transformations

The function of vitamin B_{12} as the precursor of an hypothetical co-factor involved in biochemical transformations is much more obscure than is the role of CF. The complicated picture in the pernicious anemia of man has already been described. In studies of bacterial metabolism similar contradictions exist. Thus, in certain lactobacilli unable to synthesize the vitamin, growth can be induced (under certain conditions) by the addition of any one of several desoxyribosides of either purines or pyrimidines; on the other hand, for certain vitamin B_{12}-dependent mutants of *E. coli,* growth can be

obtained by the addition of methionine (but not by the use of the derivatives of desoxyribose). Also, there is evidence that the vitamin is involved in the synthesis of the purine ring (39). From rats and chicks excellent evidence has been obtained (40) that vitamin B_{12}, either directly or indirectly, is needed for the synthesis of methionine from homocystine (by the introduction of a methyl group following conversion to homocysteine, or a derivative thereof) and, in rats, the folic acid-dependent conversion of glycine to serine also appears to require vitamin B_{12}. This is shown by the findings that vitamin B_{12} is not needed for the formation of the methyl groups of choline from the β-carbon of serine (41), but is required for utilization of the α-carbon of glycine in a similar manner (41, 27). No single common feature has been discerned amongst these observations, but there is now much speculation concerning the possibility that homocysteine may play a key role in these reactions, since it has been found remarkably to increase the synthesis not only of methionine, but also of purines, and its role in the synthesis of cystathionine and thus of cysteine and probably of serine is evident. Conceivably, vitamin B_{12} might be involved in a reaction concerned with the formation or utilization of a homocysteine-like compound or the reaction of a derivative of it with a one-carbon moiety. Such a possibility may be suggested also by the reaction discovered by Cantoni (42), in which the enzymatic "activation" of methionine by adenosine triphosphate (ATP) was shown to consist in the formation of a compound termed S-adenosyl methionine, with a labile S-linked methyl group. Whether analogous derivatives of homocysteine, possibly with a formyl or $-CH_2OH$ group linked to the sulfur, play a biological role is an attractive speculation.

Role of Intrinsic Factor in the Utilization of Vitamin B_{12}

In the early part of this article reference was made to the fact that in pernicious anemia vitamin B_{12} fails to be absorbed primarily because of a deficiency of a substance (intrinsic factor) secreted in gastric juice. The role of the intrinsic factor in promoting the removal of the vitamin from the intestinal content has been demonstrated by the use of radioactive ($cobalt^{60}$) vitamin B_{12}. With the labeled vitamin it was possible to show that either treated or untreated patients with pernicious anemia lost in their feces almost all of the radioactivity of even such minute amounts as 0.5 microgram of ingested vitamin. However, with co-administration of radioactive vitamin B_{12} and active sources of intrinsic factor, only small amounts of radioactive material appeared in the feces (2). This effect of intrinsic factor not only shows that the substance is involved in making vitamin B_{12} available for

absorption, but also has permitted the use of relatively healthy, treated individuals in assaying materials for intrinsic factor activity. Previously the greatest difficulty was experienced, in work directed toward the purification of the intrinsic factor, because of the scarcity, and complications in the use for assay purposes, of extremely ill patients with pernicious anemia.

Wide publicity has been accorded various reports (43-46) of the ability of gastric juice and gastric extracts to bind vitamin B_{12} in such a manner as to render it unavailable for utilization by micro-organisms; however, these observations have not led to a practical assay for intrinsic factor. Although it may well be that intrinsic factor indeed does "bind" vitamin B_{12}, this property unfortunately is demonstrated also by associated substances of gastric tissue which have little or no intrinsic factor activity (47, 48). Hoff-Jorgensen has reported (46) that the distribution in extracts of the alimentary tract of vitamin B_{12}-binding substances, as determined with a mutant of *E. coli,* agrees with published evidence concerning the distribution of intrinsic factor. It has been suggested that the binding of vitamin B_{12} by certain intestinal bacteria is responsible for the failure of patients with pernicious anemia to utilize the ingested vitamin. Whether these findings will prove to be related to the function of intrinsic factor cannot yet be determined.

It has been reported (1, 47, 48) that highly potent concentrates of intrinsic factor have been prepared from powdered gastric tissue and from gastric juice (with some fractions a dose of less than 1 mg. daily was required for activity). Material that conceivably may approach homogeneity has been described by Ungley and his associates (49), who obtained it in small amounts by the electrophoresis of gastric juice on paper strips. With isolation of large amounts of the factor, which is believed to be a polypeptide rather than a protein (49), detailed studies of its mechanism of action will be possible.

At the present time it does not appear likely that intrinsic factor will have legitimate practical applications of any great significance. Only in pernicious anemia is there a known deficiency of the factor, and the number of such individuals not only is relatively small, but it is probable that they may be treated more wisely by the occasional injection of vitamin B_{12}, at which time adequacy of therapy can be assured by proper medical examination. If oral therapy is desirable it appears that relatively massive oral dosage with vitamin B_{12} (1 to 3 mg.) will lead to the absorption of a sufficient amount of the ingested vitamin to accomplish the desired end. This may be preferable to the therapeutic use of expensively prepared concentrates of a heat-labile polypeptide or protein, in conjunction with vitamin B_{12} in doses of a few micrograms.

The Interrelated Action of CF and "Hematopoietic Factor" in the Function of Bone Marrow

The important findings of Callender and Lajtha (4), previously referred to, may now be discussed in the light of the complete picture. These authors have investigated the response *in vitro* to hematopoietic agents of megaloblastic bone marrow (from patients with pernicious anemia in relapse). Their findings show that on aseptic incubation in diluted normal serum the megaloblasts of the bone marrow disappear, while in diluted serum from pernicious anemic patients in relapse the megaloblastic cells are preserved, or "ripen" less rapidly. When to the latter incubation mixture are added relatively large amounts of PGA, or very minute amounts of CF, the megaloblasts disappear rapidly. That marrow has a weak but definite ability to convert PGA to CF has been shown (11). Accordingly, it is postulated, in keeping with other findings described in this article, that CF is involved critically in the maturation of the megaloblast, and, in fact, that the megaloblast appears as the result of a deficiency of CF (4).

The results with vitamin B_{12} are equally intriguing. Whereas addition of the vitamin *per se* is without effect, a combination of vitamin B_{12} and a small amount of sterile filtered normal human gastric juice causes the disappearance of megaloblasts; gastric juice by itself is without effect. It would appear, then, that in order to explain the activity of *parenterally* administered vitamin B_{12} in pernicious anemia one must postulate the existence of an extra-gastric intrinsic factor involved in some way in the utilization of vitamin B_{12} by the cells of the bone marrow. It has been suggested that the combination yields a new "hematopoietic factor" (4). Accordingly, it is necessary to view pernicious anemia as a disease resulting from a *local* deficiency of intrinsic factor in the gastro-intestinal tract as a result of which *ingested* vitamin B_{12} fails to be absorbed. However, production of intrinsic factor elsewhere in the body would appear to be unimpaired, at least in the majority of patients. Further, in order to account for the direct action of CF, and for the development of megaloblasts in the bone marrow despite the ingestion by pernicious anemic individuals of precursors of CF, it has been postulated that in this disease an inhibitory factor is present which interferes with the action of normal physiologic amounts of CF or its derivatives (4). That such may be the case is suggested also by experiments with marrow *in vitro* in which it has been found that high concentrations of serum from patients with pernicious anemia may cause an *increase* in the number of megaloblasts during incubation. As the serum is diluted with a modified Ringer solution this "megaloblastogenic" action disappears. Thus, it could be that in pernicious anemia there accumulates, as a result of a deficiency of vitamin

B_{12}, a substance which interferes with the effective action of CF. According to this view, the parenteral administration of vitamin B_{12} (in conjunction with extragastric intrinsic factor), results in the neutralization or inactivation of the inhibitor, and CF is enabled to support a normal pattern of red blood cell formation. On the other hand, by supplying PGA (or CF), in more than physiologic amounts, the effect of the postulated inhibitor may be circumvented and normal maturation can be obtained.

These concepts, proposed by Callender and Lajtha (4), are of great interest since they appear to be in agreement with much that we know, and the essential findings have been confirmed (49). However, additional investigation is needed, particularly in connection with the hypothesis that an inhibitor of CF plays a critical role in the pathogenesis of pernicious anemia, in order to clarify further our understanding of the metabolic defects of this disease.

In conclusion, it is quite clear that an agent concerned with the formation of normal red blood cells can be formed from pteroylglutamic acid or its derivative, citrovorum factor (folinic acid), and that a chronic deficiency of this agent leads to a macrocytic anemia characterized by the appearance of megaloblasts in the bone marrow. In some manner not yet elucidated vitamin B_{12} apparently is involved in the formation, stabilization or activation of this agent (at least in man). The citrovorum factor, probably by serving as the precursor of a coenzyme concerned with the transport of one-carbon metabolic intermediates, is critically involved in the synthesis and degradation of a large number of compounds essential for the reproduction of a wide variety of cells, and particularly of the cells of the hematopoietic system.

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NEWS DIGEST

Vitamin C and Cholesterol Synthesis

As our knowledge of nutrition, biochemistry and medicine expands, it is becoming increasingly clear that the so called "degenerative diseases" offer a great challenge that has so far defied solution.

Arteriosclerosis, while not necessarily a degenerative disease in so far as it affects many relatively young people as well as the aged, is a most controversial subject especially as to its etiology. This is not surprising considering the fact that in the absence of adequate information confusion always reigns supreme.

The fact that a high degree of correlation is seen between hypercholesterolemia and arteriosclerosis has tended to incriminate cholesterol as a causative factor in this disease. The discovery of "macromolecules" in blood, composed of protein and lipid materials (including choles-

terol), and the relation of the quantity of circulating macro-molecules to arteriosclerosis has served to broaden our knowledge and make us even more aware of the complexities of the problem.

It would appear that the actual amount of dietary cholesterol is not of major importance in determining the level of this substance in circulating blood, since numerous studies have shown that cholesterol can be synthesized in the body from the simple and readily available two carbon unit, acetate. It is also of significance that the level of circulating cholesterol can be markedly influenced by the amount of dietary fat even with no cholesterol in the diet, (ie) cholesterol levels tend to be low on low fat intakes and to increase as the fat intake increases.

Recent experiments reported by C. G. King and co-workers (J.A.C.S., *75*, 2020, 1953) bring additional light on the role of nutrition in cholesterol synthesis by the body. When acetate, labelled with radioactive carbon was administered to guinea pigs, both normal and scorbutic, it was observed that the vitamin C deficient guinea pig synthesized adrenal cholesterol about 6 times as fast as the normal nonscrobutic animals. With this finding it is clear that at least 2 vitamins are concerned with the body's ability to synthesize cholesterol viz.—pantothenic acid in the form of coenzyme A and vitamin C.

It is of course well known that a variety of stress conditions evoke in the adrenal of normal animals a change in adrenal ascorbic acid and cholesterol concentration similar to that obtained following the injection of adrenocorticotrophic hormone (ACTH). The adrenal response is characterized by a marked fall of both adrenal cholesterol and ascorbic acid concentrations. It might be further noted that in pantothenic acid deficient animals this response is no longer evident following the administration of ACTH.

Oesterling and Long (Science, 113, 241, 1951) found that while normal guinea pigs showed the usual drop in adrenal ascorbic acid after stress, the scorbutic animals did not, partly because their adrenal ascorbic acid levels were greatly depleted by the lack of dietary vitamin C.

On the other hand, these workers found that in early stages of scurvy, even though there was very little ascorbic acid in the adrenal, the cholesterol levels were actually higher than in the normal control animals. "The adrenal at this stage, although 95% depleted of vitamin C, responds to injection of ACTH by showing a 42% reduction in cholesterol concentration after 6 hours." In the late stages of advanced scurvy, however, the adrenal cholesterol levels were significantly lower than that of the controls.

It is quite evident that a relationship exists between hormone activity, vitamin C concentration of the adrenal and cholesterol synthesis. It will undoubtedly take considerably more work to clarify this relationship and to establish its significance if any to the problem of arteriosclerosis.

ABOUT THE AUTHOR

Arnold Welch, the author of the article, Nutrition and Hematopoiesis, is particularly qualified to write on this subject as the result of his numerous and significant research efforts in this area of biochemistry and nutrition. *Borden's Review of Nutrition Research* is justly proud to add his name to the list of distinguished contributors to this Review.

Dr. Welch was born November 7, 1908 in Nottingham, New Hampshire and received his elementary school training at Lynn, Massachusetts and Dade City, Florida. His advanced training was started at the University of Florida where he received the B.S. degree in 1930 and the M.S. degree in 1931. After a summer session at the University of Minnesota he enrolled for his graduate studies at the University of Toronto and received the Ph.D. degree from that institution in 1934. While at the University of Toronto, Dr. Welch was priviledged to work under the direction of Professors V. E. Henderson, C. H. Best, and Arthur Wynne.

After completion of his Ph.D. degree, Dr. Welch journeyed to St. Louis, Mo. where at Washington University he became successively assistant and instructor in Pharmacology. At the same time, Dr. Welch enrolled in the Washington University School of Medicine which awarded him the M.D. degree in 1939. It was at Washington University that Dr. Welch came under the stimulating guidance of Prof. Carl F. Cori who provoked further his already awakened interest in biochemistry.

Subsequent to a period of service with Sharp and Dohme, where he became director of research, Dr. Welch returned to the academic fold to become Professor of Pharmacology at Western Reserve University, which post he will hold until July 1, 1953, when he will assume the responsibility of Professor and Chairman of the Department of Pharmacology at Yale University, New Haven, Connecticut. The cycle is completed from New England back to New England.

Dr. Welch's service to the scientific community includes appointments to the National Research Council, National Science Foundation, and the U. S. Public Health Service. As a Fulbright Senior Research Scholar, Dr. Welch just completed 6 months of study at Oxford University, England.

The numerous publications of Dr. Welch and his membership in many scientific and honorary societies serve to emphasize the important place that Dr. Welch holds in the scientific scene. *Borden's Review of Nutrition Research* takes this opportunity to congratulate him on his past accomplishments and to wish him success in his new post at Yale University.

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